U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
STOP 3630
WASHINGTON, DC 20090-3630

CHAPTER 19

ROSA® Aflatoxin (Quantitative) TEST KIT

19.1 GENERAL INFORMATION

The ROSA® Aflatoxin (Quantitative) test kit uses lateral flow test strip technology that provides quantitative results. **The test kit is limited to providing aflatoxin measurements between 5 – 100 ppb.** Accurate aflatoxin measurements from **101 - 400 ppb** can be obtained by performing a supplemental analysis involving a diluted extract.

19.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the ROSA® Aflatoxin (Quantitative) test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (Reagent grade or better) and 30 percent water.

a. Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

b. Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

19.3 PREPARATION OF TESTING MATERIALS

NOTE: A Negative and Positive Control must be run daily to verify performance of equipment and test strips.

a. Negative Control.

Add 100 µl of 70% methanol solution to 1.0 ml of AFQ Buffer to prepare Negative Control Diluted Extract. **Negative Control must read less than 3 ppb.**

b. Positive Control.

Prepare the Positive Control by adding 3.0 ml of deionized or distilled water and 300µl of 70% methanol to Aflatoxin B1 Control. Mix thoroughly. **Positive Control must read between 10-30 ppb.**

NOTE: Store at 32-45 °F for up to one week, or freeze at -4 °F for 2 months.

c. Equipment Preparation.

(1) Incubator must be at 45±1°C (temperature indicator is green).

(2) Incubator must be clean and level.

d. AFQ Dilution Buffer.

Predispense 1.0 ml of AFQ Dilution Buffer into a micro-centrifuge tube for each sample to be tested.

e. Test Strips.

(1) Remove ROSA® moisture resistant container from the refrigerator and allow it to reach room temperature to limit condensation.

(2) Remove only the number of strips to be used and return container to 32-45 °F storage. Strips are stable at room temperature for at least 12 hours.

NOTE: If blue desiccant packets turn white or pink, test the strips with Negative and Positive Controls before continued use.

19.4 EXTRACTION PROCEDURES

a. Procedures for Extraction of Corn, Cracked Corn, Corn Germ Meal, Corn Gluten Meal, Corn Screenings, Milled Rice, Rough Rice, and Sorghum

(1) Transfer 50 grams of ground sample into a clean extraction container.

(2) Add 100 ml of the (70/30) methanol/water extraction solvent.

(3) Shake or blend for 1 minute. Allow sample to settle for 1 minute to obtain a clear sample extract.

(4) Continue to sample preparation step.

b. Procedures for Extraction of Corn Flour, Corn Meal, Corn Soy Blend, Flaking Corn Grits, Popcorn, and Wheat

(1) Transfer 50 grams of ground sample into a clean extraction container.

(2) Add 100 ml of the (70/30) methanol/water extraction solvent.

(3) Shake or blend for 1 minute. Allow sample to settle for 1 minute to obtain a clear sample extract.

(4) Pass 1 ml of clear sample extract through a Minisart RC 15 filter syringe.

(5) Continue to sample preparation step.

c. Procedures for Extraction of Soybeans and Distillers Dried Grains

(1) Transfer 50 grams of ground sample into a clean extraction container.

(2) Add 150 ml of the (70/30) methanol/water extraction solvent.

(3) Shake or blend for 1 minute. Allow sample to settle 1 minute to obtain clear sample extract.

(4) Continue to sample preparation step.

NOTE: If particles are present after settling, filter or centrifuge to clarify sample extract. **To Filter:** funnel the extract through Whatman 2V (or equivalent) filter paper into a labeled collection container. **To Centrifuge:** transfer 1.0-1.5 ml of sample extract to a labeled micro-centrifuge tube and centrifuge for 10 seconds. Clarified extract is now ready for testing.

19.5 TEST PROCEDURES

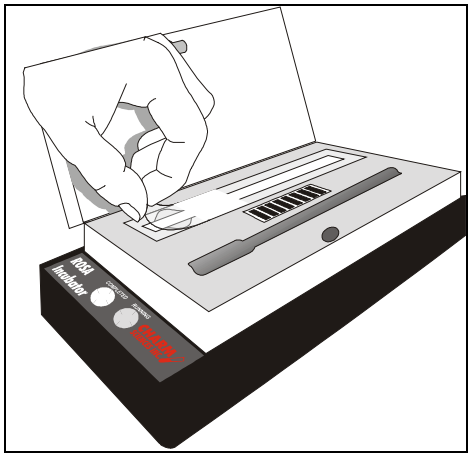
a. Sample Preparation

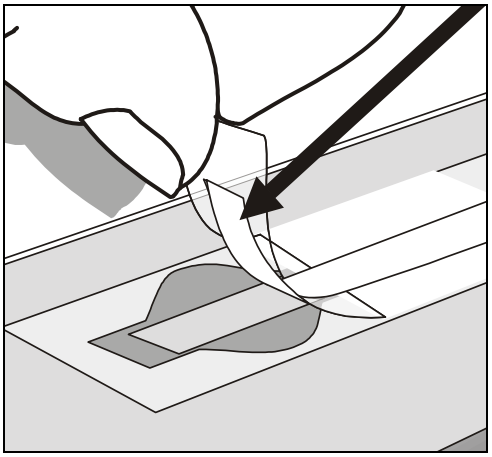
(1) Pipet 100 µl of filtered clarified extract to a predispensed (1.0 ml AFQ Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract.

(2) Label the test strip to identify sample.

(3) Open the incubator lid and place test strip in the ROSA-M Incubator with the lat side facing upward.

(4) While holding the strip flat on the incubator, use tab to peel tape back to the indicated line exposing the sample pad. Avoid bending back the white wick and sponge under the tape.

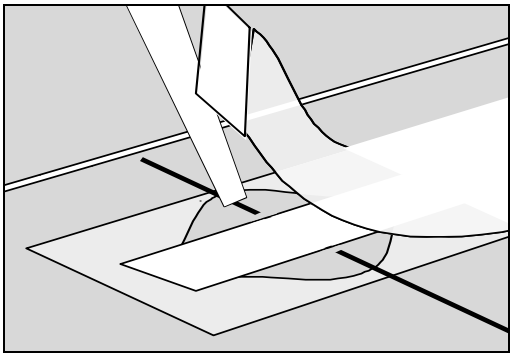




c. Sample Analysis.

(1) Pipet 300 µl of diluted extract into the side of the side of the strip sample compartment at the position indicated by the black line on the incubator.

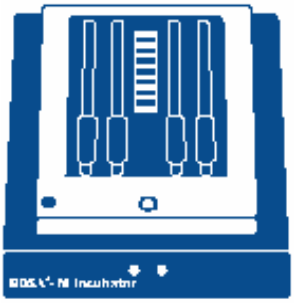
NOTE: Pipet very slowly.



(2) Reseal the tape over the sample pad compartment. When testing multiple samples, complete the peel, pipet, and reseal steps on each strip before going to the next strip.

NOTE: Add diluted extract to all strips within 1 minute. If a quad incubator is used, 4 samples can be incubated simultaneously.

(3) Close lid on the incubator and tighten the latch. The solid red timer light will automatically start when the lid is closed.



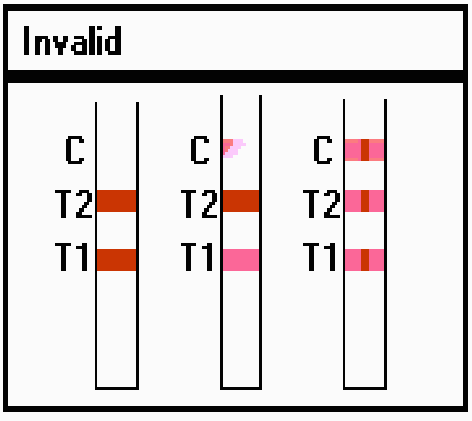
LF-INC4-45D: Quad incubator, 3-minute timer with display, set for 45° C for Test Strips

(4) Incubate for 10 minutes. After the incubation step is complete, a beeper will sound and the yellow "test complete" light will begin to flash.

(5) Remove strips and interpret the results. **Strips must be removed from the incubator and read within 2 minutes of incubation completion.** After strip removal, lower but do not latch the incubator lid.

c. Visually Interpreting the Lateral Flow Test Strip.

A test is **invalid** if a Control Line (C) is missing, smeared, or uneven, or if either Test Line is uneven. It is invalid if the diluted extract is obscuring either the Control (C) or Test Line (T) or if the beads do not flow past Test and Control Lines. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh dilution) should be made and tested using another strip.



d. Interpreting the Lateral Flow Test Strip using the ROSA-M Reader.

(1) Insert a clean valid test strip into the ROSA-M Reader. Slide the strip into the slot, with the sample compartment in the up position, until it stops.

LF-ROSA READER-M: ROSA-M Reader supplied with calibrators.

(2) Read result on **AFLA** Channel (3-Line Mode) using the appropriate MATRIX on the ROSA-M Reader. If desired, enter **Sample** and/or **Operator.** Press **ENTER** to read.

NOTE: Use the following table to determine the Matrix number to be used.

Matrix 0	**corn**
Matrix 1	**cracked corn, corn flour, corn germ meal, corn gluten meal, corn meal, corn screenings, corn soy blend, flaking corn grits, milled rice, rough rice, popcorn, sorghum, wheat**
Matrix 3	**distillers dried grains, soybeans**

(3) **READING:** The number dispayed is the concentration of aflatoxin (ppb) in the sample. Readings greater than 100 ppb must be diluted and retested.

19.6 REPORTING AND CERTIFYING TEST RESULTS

a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

b. Sample results over 100 ppb are reported as >100 ppb unless a supplemental analysis is performed.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

19.7 SUPPLEMENTAL ANALYSIS

NOTE: The supplemental analysis is limited to providing results between 101 – 400 ppb.

a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 100 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 100 ppb, the sample extract must be diluted so that a value between 100 and 400 ppb is obtained.

b. Dilution for Corn, Cracked Corn, Corn Flour, Corn Germ Meal, Corn Gluten Meal, Corn Meal, Corn Screenings, Corn Soy Blend, Flaking Corn Grits, Milled Rice, Rough Rice, Popcorn, Sorghum, and Wheat.

If the original analysis reported the aflatoxin value at greater than 100 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Predispense 1.0 ml AFQ Dilution Buffer to a clean, labeled micro-centrifuge tube.

(2) Pipet 100 µl of diluted extract from step 19.5 a (1) to the micro-centrifuge tube.

(3) Proceed to sample analysis.

(4) Read results on AFLA Channel (3-Line Mode) on Matrix 03. The number displayed is the concentration of aflatoxin (ppb) in the sample.

NOTE: Sample readings of less than 100 ppb or greater than 400 ppb (+400 ppb on the display) are not within the test range. Results less than 100 ppb can be retested using the standard procedure.

c. Dilution for Soybeans and Distillers Dried Grains

If the original analysis reported the aflatoxin value at greater than 100 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Predispense 0.6 ml AFQ Dilution Buffer to a clean, labeled micro-centrifuge tube.

(2) Pipet 100 µl of diluted extract from step 19.5 a (1) to the micro-centrifuge tube.

(3) Proceed to sample analysis.

(4) Read results on AFLA Channel (3-Line Mode) on Matrix 03. The number displayed is the concentration of aflatoxin (ppb) in the sample.

NOTE: Sample readings of less than 100 ppb or greater than 400 ppb (+400 ppb on the display) are not within the test range. Results less than 100 ppb can be retested using the standard procedure.

19.8 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used extraction mixing jars and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used test strips and pipettes, for at least 5 minutes.

Pour off the liquid down the drain and place the materials in a garbage bag and discard.

19.9 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), dispose of any remaining liquid filtrate in the chemical waste container. Discard the sample slurry (ground material) into a plastic garbage bag for disposal.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the slurry (ground portion) remaining in the sample extraction jar must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, pour approximately 50 ml of bleach solution into the sample extraction jar and shake to mix with the sample slurry. After the slurry and bleach solution separate, handle the bleach rinse filtrate as a non-hazardous solution and dispose of by pouring the liquid down the down. Discard the sample slurry (ground portion) paper into a plastic garbage bag for disposal.

19.10 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

Kits can be purchased that contain 20, 100, or 500 strips and include Control and AFQ Dilution Buffer.

(1) LF-APN-20 –

(a) 1 package containing 20 ROSA® strips packed in a moisture-resistant container.

(b) 1 Aflatoxin B1 20 ppb Control.

(c) 1 AFQ Dilution Buffer.

(2) LF-APN-100 –

(a) 1 package containing 100 ROSA® strips packed in a moisture-resistant container.

(b) 1 Aflatoxin B1 20 ppb Control.

(c) 1 AFQ Dilution Buffer.

(3) LF-APN-500 –

(a) 5 packages containing 100 ROSA® strips packed in a moisture-resistant container.

(b) 5 Aflatoxin B1 20 ppb Controls.

(c) 5 AFQ Dilution Buffers.

b. Materials Required but not Provided:

(1) Sample grinder.

(2) Balance.

(3) Methanol - Reagent grade or better.

(4) Deionized or Distilled water.

(5) Sample extraction containers.

(6) 1.0 ml pipettor and pipette tips.

(7) 300 µl pipettor and pipette tips.

(8) 100 µl pipettor and pipette tips.

(9) 25 ml graduated cylinder.

(10) 1.5 ml micro-centrifuge tubes.

(11) Minisart RC 15 filter syringe.

c. Optional Equipment and Supplies:

(1) Mini-centrifuge

(2) Whatman 2V filter paper or equivalent.

(3) Filter funnel.

19.11 STORAGE CONDITIONS

a. Storage Conditions.

Test kits should be refrigerated between 32°- 45°F.

b. Precautions.

(1) Do not use the test kits beyond the noted expiration date.

(2) Prolonged exposure to high temperatures may adversely affect the test results.

(3) Do not open the desiccated canister until ready to use the strips.